

## IN VITRO ACTIVITY OF PARIJOTO FRUIT EXTRACT (*Medinilla speciosa* B.) FOR REDUCING BLOOD GLUCOSE

### *Aktivitas In Vitro Penurun Gula Darah dari Ekstrak Buah Parijoto (Medinilla speciosa B.)*

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#### INFO ARTIKEL

##### **Article history:**

Diterima: 11 April 2019

Direvisi: 19 Juli 2019

Disetujui: 30 April 2020

##### **Keywords:**

Flavonoids; hyperglycemia;  
ethanol fraction; Nelson-  
Somogyi

##### **Kata kunci:**

Flavonoid; hiperglikemia;  
fraksi etanol; Nelson-  
Somogyi

#### ABSTRACT/ABSTRAK

Diabetes mellitus is one of the highest causes of death in the world, with symptoms of increased blood glucose levels (hyperglycemia). One of the efforts made as a treatment and prevention of DM is through complementary therapy using natural ingredients. Parijoto fruit (*Medinilla speciosa*) contains flavonoid compounds potential to reduce glucose levels. The study aimed to analyze the effectiveness of ethanol extracts and its fractions of parijoto fruit (EEBP) to decrease glucose levels in vitro. The study was conducted at the Ngudi Waluyo University Laboratory in April to August 2018. Identification and screening of flavonoid compounds from ethanol extracts and its fractions of parijoto fruit were performed qualitatively and by thin-layer chromatography (TLC). *In vitro* testing for antidiabetic activity of EEBP was performed with non-enzymatic reaction by Nelson Somogyi method (ethanol extract and its fractions). Experiment was arranged in a randomized block design, with 12 treatments and three replications. The concentrations of parijoto fruit tested were 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. The results of qualitative identification and TLC showed that the ethanol extract and its fraction of EEBP contained flavonoid compounds. At a concentration of 30 ppm, EEBP was able to reduce glucose levels by 42.43 %, while the ethanol fraction decreased glucose levels by 83.38 %. The results showed parijoto fruit ethanol extract and its fractions were sources of antidiabetic. Hence its pharmacologically effect was necessary to be further studied.

*Penyakit diabetes melitus (DM) merupakan salah satu penyebab kematian tertinggi di dunia dengan gejala peningkatan kadar glukosa (hiperglikemia) dalam darah. Salah satu upaya yang dilakukan sebagai pengobatan dan pencegahan DM adalah melalui terapi komplementer menggunakan bahan alam. Buah parijoto (Medinilla speciosa) mengandung senyawa flavonoid yang berpotensi dalam menurunkan kadar glukosa. Tujuan penelitian adalah menganalisis keefektifan ekstrak dan fraksi etanol buah parijoto (EEBP) terhadap penurunan kadar glukosa secara in vitro. Penelitian dilaksanakan di Laboratorium Universitas Ngudi Waluyo sejak April sampai Agustus 2018. Identifikasi dan skrining senyawa flavonoid dari ekstrak dan fraksi etanol buah parijoto dilakukan secara kualitatif dan kromatografi lapis tipis (KLT). Aktivitas antidiabetes EEBP diuji secara in*

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DOI : <http://dx.doi.org/10.21082/bullitro.v31n1.2020.31-39>

0215-0824/2527-4414 @ 2017 Buletin Penelitian Tanaman Rempah dan Obat

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Accreditation Kemenristekdikti Number : 30/E/KPT/2018

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*vitro dengan metode non-enzimatis Nelson-Somogyi (ekstraksi dan fraksi etanol) menggunakan rancangan acak kelompok, 12 perlakuan dengan tiga ulangan. Konsentrasi buah parijoto yang diuji adalah 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, dan 60 ppm. Hasil identifikasi kualitatif dan KLT menunjukkan bahwa ekstrak dan fraksi etanol EEBP mengandung senyawa flavonoid. Pada konsentrasi 30 ppm, EEBP mampu menurunkan kadar glukosa sebesar 42,43 %, sedangkan fraksi etanolnya menurunkan kadar glukosa 83,38 %. Penelitian ini menunjukkan bahwa ekstrak dan fraksi etanol buah parijoto berpotensi sebagai sumber antidiabetes, namun perlu diteliti lebih lanjut secara farmakologis.*

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## INTRODUCTION

Diabetes (DM) is a long-term dysfunction in the body's organs, especially concerning the function of the kidneys, eyes, nerves, heart, and blood vessels (American Diabetes Association 2014). The epidemiological studies showed that the main factor causing DM was increasing blood glucose levels or hyperglycemia (Jakus & Rietbrock 2004; Prashanto K. Das *et al.* 2011). The prevalence of DM tends to increase every year in both productive and non-productive ages (Shaw *et al.* 2010). In 2018, the prevalence of DM sufferers between the ages of 35-44 years was 1.1 %, 45-54 years was 3.9 %, and it increased by 6.4 % at the age of 55-64 years (RISKESDAS 2018). The effort to prevent DM is by reducing the prevalence rate. Early prevention can be done by always maintaining the consistency of the pattern and quality of food. The use of oral drugs does not always have a positive impact on DM treatment. Oral medication use often has side effects that lead to other symptoms causing complications (Fadillah 2014; Fatimah 2015).

The use of natural ingredients as traditional medicine is one alternative treatment for DM. Phenolic and flavonoid compound content in most natural ingredients has the potential as antidiabetic by decreasing glucose index (Rao *et al.* 2010). Plant belongs to *Medinilla* genus, which reported to have secondary metabolite compounds as antidiabetic, is *Medinilla speciosa* B, known as parijoto fruit. Parijoto fruit harvested at one-month-old has the highest content of phenolic compounds and antioxidant activity compared to two and three months old (Ameliawati 2018). Moreover, the methanol extract of parijoto fruit

reported to contains flavonoid compounds (Tussanti dan Johan 2014); (Sa'Adah *et al.* 2018). Flavonoid compounds can reduce glucose levels *in vitro* by binding the –OH group to glucose. The remaining glucose that did not bind the –OH groups can be observed spectrophotometrically visible by the Nelson-Somogyi method (Somogyi 1951; Razak *et al.* 2012).

The activity of active compounds of a natural substance could increase through the fractionation process based on the compounds' nature. Fractionation aims at extracting the desired compound, eliminating other compounds that interfere with, and concentrating the content of compound. Several studies have shown that the fractionation process can improve the functional specifications of a secondary metabolite compound in natural substances (Sasidharan *et al.* 2011). The purpose of this study was to analyze the effectiveness of ethanol extracts and its fractions of parijoto fruit to decrease glucose levels *in vitro*.

## MATERIALS AND METHODS

The study was conducted from April to August 2018 at the Phytochemical Laboratory for the extraction and fractionation of parijoto fruit. The reduction of glucose levels *in vitro* analyzed with a non-enzymatic method using the Nelson-Somogyi procedure at the Instrument Laboratory of Ngudi Waluyo University. The experiment arranged in a randomized block design (RBD), 12 treatments, and three replications. The treatments tested were six concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm) of both ethanolic extract and its fraction of parijoto fruits.

## Parijoto fruit

Parijoto fruit (*Medinilla speciosa* B.) having purplish color and sour taste collected from a farmer at Colo Village, Dawe District, Kudus Regency, Central Java. Three kilograms of the fruits were cleaned from dirt and debris, washed with running water, and then air-dried. The fruits were then chopped to quicken the drying process. The dried fruit was ground and sieved using a 60 mesh sieve to obtain a homogeneous powder.

## Extraction and fractionation

Extraction was performed using the maceration method with 96% technical grade ethanol as solvent (Azwanida 2015). The dried pieces of parijoto fruits were macerated with ethanol (1:10) for two days and remacerated for 24 hours using the same method. The resulting extract was then evaporated with a rotary evaporator (RE 100-Pro) to produce the viscous extract. The resulting viscous extract was assumed to contain secondary polar, semi-polar and non-polar metabolites.

The parijoto fruit ethanol extract (10 g extract) was fractionated using liquid-liquid fractionation. The first stage of the fractionation process used n-hexane solvent, followed by ethyl acetate, and the last was by ethanol 96 % (1:10). The ethanol phase was taken and evaporated with a rotary evaporator until a viscous phase was obtained. The fraction was the result of the extract partitioned with polar solvents (ethanol) to get polar compounds.

## Qualitative screening and identification of flavonoids

Qualitative identification of active compounds was analyzed by thin-layer chromatography (TLC) to screen the phytochemical characteristics of secondary metabolites such as flavonoid, saponins, tannins, and alkaloids following (Sheela 2011) method. Flavonoid identification used the mobile phase of n-butanol, acetic acid, and distilled water (3: 1: 1) and the stationary phase of silica gel GF<sub>254</sub>. The TLC test was initiated with the saturation of the

mobile phase in the vessel or chamber to obtain atmospheric homogeneity to minimize the evaporation of solvents from the TLC plate (stationary phase) during development. It aimed to separate the spot entirely (Gwatidzo *et al.* 2018; Bhawani *et al.* 2010). The TLC process was continued by spotting the ethanol extract of the parijoto fruit onto a silica plate and developed in a vessel that contained a mobile phase. The spot appearance to confirm the presence of flavonoid compound was examined using ammonia vapor to provide alkaline condition (Mu'awwanah & Ulfah 2015).

## In vitro glucose reduction test

The effect of ethanol extract and fractionation of the parijoto fruit in reducing glucose levels in vitro was examined following Nelson-Somogyi method (Somogyi 1951) using D-glucose. The principle of the Nelson-Somogyi's operation was that the flavonoid compounds in the extract and ethanol fraction of the parijoto fruit would bind glucose and form a flavonoid-glucose complex bond. In contrast, the remaining glucose, which is not bound by flavonoids, will be reduced and bound by arsenic-molybdate compounds in the Nelson-Somogyi solution. Thus, a UV-Vis spectrophotometer can read the colored mixture. The smaller the remaining glucose that was not bound by flavonoids, the higher the ability of these compounds to reduce glucose levels.

The first step in the glucose reduction testing was determining the maximum wavelength in the range of 700-780 nm and proceed with determining operating time. The research was arranged in a complete randomized block designed, repeated three times. The parijoto fruit ethanol extract and its fractions were formulated with a series of concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. A standard glucose solution of 80 ppm was added to the series ethanol extract concentration and its fraction of parijoto fruit. Thereafter, Nelson reagent and arsenic-molybdate were added to form a complex colored solution. Data analysis used the following equation :

$$\text{Percentage of glucose reduction} = \frac{\text{Initial level} - \text{Final level}}{\text{early content}}$$

Note/Keterangan :

Initial level/level awal = standard glucose level/level glukosa standar.

Final levels/level akhir = levels after addition of ethanol extracts and its fractions of the parijoto fruit/level glukosa setelah penambahan ekstrak dan fraksi etanol buah parijoto.

### Data analysis

The data were analyzed using analysis of variant and tested further with Tukey (HSD) at 5% if there were significant differences.

## RESULTS AND DISCUSSION

### Parijoto fruit extraction and fractionation

The yield of parijoto ethanol extract was 8.66%. This yield was higher than the results of maceration using methanol conducted by (Sa'adah *et al.* 2017; Sa'adah *et al.* 2018). It was likely due to the 96% ethanol solvent being able to attract more active compounds present in the extract of the parijoto fruit. (Arifianti *et al.* 2014) suggested the ideal solvent in the maceration process was alcohol or a mixture of lye and water. The ethanol-water mixture ratio of 7: 3 was more suitable for the extraction of roots, stems, or wood plant materials. However, the alkaline-water mixture (1: 1) was more appropriate for removing chlorophyll, resin, and polymer. The yield of the parijoto fruit extract fraction was 42.91 %. Fractionation with ethanol solvents was expected to increase the flavonoid content.

### Qualitative screening and identification of flavonoids

Phytochemical screening results of fruit extract and ethanol fraction of parijoto fruit showed the presence of flavonoid, saponins, tannins, and alkaloids (Table 1). Based on the results of the analysis using the Wilstater method, the flavonoid compounds in the sample were identified as orange after the addition of Mg and HCl powders. It occurred because Mg and HCl powders reduced the benzopiron nucleus contained in the flavonoid structure, resulted in color change

Table 1. The result of phytochemical-qualitative screening of ethanol extract and its fraction of parijoto fruit.

Tabel 1. Hasil skrining fitokimia ekstrak dan fraksi etanol buah parijoto secara kualitatif.

Active compound	Identification method	Result	
		Ethanol extract	Ethanol fraction
Flavonoid	Wilsttater Cyanidin test	+	+
Saponin	Forth test	+	+
Tannin	Ferric chloride test	+	+
Alkaloid	Dragendroff's reagent	+	+

Note/Keterangan :

+ (positive reaction/reaksi positif).

into orange (Sheela 2011). These results confirmed the flavonoid presence in the ethanol extract and its fraction of parijoto fruits.

The presence of flavonoids in the ethanol extract and its fraction of parijoto fruit was further confirmed by TLC analysis (Table 2). The TLC separation on the ethanol extract and fraction showed the presence of brown spots when exposed to UV254, indicated the presence of flavonoid. After being evaporated with ammonia, the flavonoid compounds appear to be a greenish-yellow color, as reported by (Mu'awwanah & Ulfah 2015). Gwatidzo *et al.* (2018), also affirmed that the presence of flavonoids in natural material samples was marked in yellow, blue, or green, and gave a more intense yellow color after the ammonia vaporization. These results indicated that the ethanol extract and its fraction of parijoto fruit contained flavonoid compounds potential to reduce glucose levels.

### In vitro glucose reduction test

Testing of glucose reducing activity of ethanol extract and its fraction of parijoto fruit based on the Nelson-Somogyi method showed positive results, as indicated by the formation of gluconic acid compounds (Figures 1 and 2). The advantages of using the Nelson-Somogyi method to measure the glucose level were its selectiveness and control easiness during measurement (Razak *et al.* 2012). Gluconic acid formation indicated

glucose content reduction. The Nelson reagent (a mixture of Nelson A and B containing copper) reacted with glucose to produce gluconic acid and cupro-oxide ( $\text{Cu}_2\text{O}$ ), characterized by the presence of a brick-red precipitate (Figure 1). The formed cupro-oxide was then reacted with arsenic-molybdate to form a copper (II) compound and a greenish-blue molybdenum complex (Figure 2). The intensity of the color produced was proportional to the amount of glucose contained in the sample. Subsequently, the samples could be observed using a UV-Vis spectrophotometer at a wavelength region of 400-800 nm (Al-Kayyis & Susanti 2016).

The treatment of ethanol extracts of parijoto fruit decreased glucose levels  $42.43 \pm 0.64\%$  at 30 ppm (Table 3). However, its ethanol fraction reduced more glucose  $83.38 \pm 0.42\%$  than

the ethanol extract, because the fraction has different polar nature (Table 3). Therefore, the flavonoid compounds in the fraction were more active in lowering glucose. The solvents in the fractionation process can increase the distribution of the separation of phenolic compounds and natural flavonoids (Irawaty *et al.* 2014; Wijaya *et al.* 2017).

Flavonoid compounds contained in ethanol extracts and its fractions of parijoto fruit were proven to reduce glucose levels. In vitro experiment showed flavonoid compounds could bind the  $-\text{OH}$  group to glucose hence decreasing glucose levels (Razak *et al.* 2012; Al-Kayyis & Susanti 2016). Flavonoid possessed a free  $-\text{OH}$  (hydroxy) group that might be bound to glucose to form a glucose-flavonoid complex. The reaction between the  $-\text{OH}$  group in flavonoids and glucose

Table 2. Identification of flavonoid compound on the ethanol extract and its fraction of parijoto fruit by using a thin layer chromatography (TLC).

Tabel 2. Identifikasi senyawa flavonoid ekstrak dan fraksi etanol buah parijoto dengan metode kromatografi lapis tipis (KLT).

Parameter	Standard	Ethanolic extract	Ethanolic fraction
$UV_{254}$ light	Brown	Brown	Brown
Ammoniac vapor	Greenish yellow	Greenish yellow	Greenish yellow

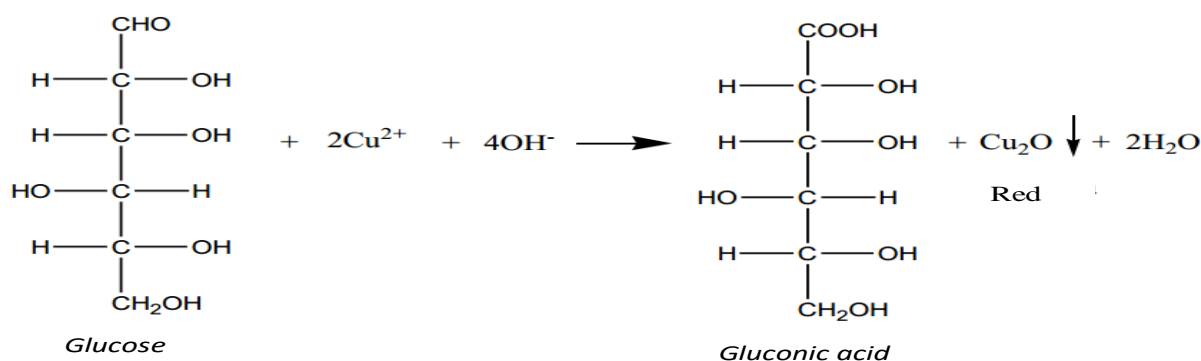


Figure 1. The reaction between Nelson reagent and glucose formed cupro-oxide.

Gambar 1. Reaksi antara pereaksi Nelson dan glukosa membentuk kupri-oksida.

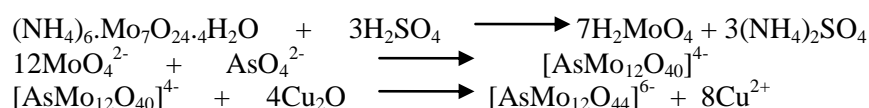


Figure 2. The formation of molybdate and cupric ( $\text{Cu}^{2+}$ ) complex.

Gambar 2. Pembentukan kompleks molybdate dan tembaga ( $\text{Cu}^{2+}$ ).

Table 3. Glucose levels decrease due to the addition of ethanolic extract and its fraction of parijoto fruit at several concentrations.

Tabel 3. Penurunan glukosa dengan penambahan ekstrak dan fraksi etanol buah parijoto pada beberapa konsentrasi.

Treatments	Concentration (ppm)	Glucose level decrease percentage (%)
Ethanolic extract of parijoto fruit	10	21.50±0.39 <sup>c</sup>
	20	37.62±0.61 <sup>d</sup>
	30	42.43±0.64 <sup>e</sup>
	40	18.29±0.41 <sup>b</sup>
	50	9.01±0.57 <sup>a</sup>
	60	7.95±0.71 <sup>a</sup>
Ethanolic fraction of parijoto fruit	10	49.46±0.28 <sup>g</sup>
	20	58.29±0.24 <sup>h</sup>
	30	83.38±0.42 <sup>i</sup>
	40	57.73±0.32 <sup>h</sup>
	50	48.02±0.29 <sup>f</sup>
	60	41.84±0.39 <sup>e</sup>

Note/Keterangan :

The number followed by the same letter in the same column were not significantly different at 5 % HSD Tukey test/Angka yang diikuti huruf yang sama pada kolom yang sama tidak berbeda nyata pada taraf uji Tukey HSD 5 %.

resulted in decreasing glucose levels in the standard solution. The remaining glucose, which was not bound by flavonoids, then reacting with the Nelson reagent to form a brick-red precipitate, which was then added to the arsenomolybdate reagent to form a greenish-blue molybdate complex (Mutiaru & Wildan 2014; Wardatun *et al.* 2016).

The treatment of ethanol extracts and its fractions of parijoto fruit showed maximum glucose levels decrease in specific concentrations. The higher the extract concentration, the higher the reduction in glucose levels. The optimal glucose levels were obtained by adding 25.01 ppm and 31.47 ppm of ethanol extract and its fraction of parijoto fruit, respectively (Figures 3 and 4). Once reaching the maximum level, the absorbance value decreased again, and the glucose level decreased again. The concentration more than 30 ppm caused saturation, represented that all free glucose has completely reacted. At this circumstance, the higher the concentration of ethanol extract and fraction added, the more concentrated the color formed. These indicated the absorbance measured by the spectrophotometer remained higher, hence lessening the activity of glucose decrease (Mutiaru & Wildan 2014; Wardatun *et al.* 2016).

The ability of flavonoids to reduce glucose levels was related to its activity as an antioxidant.

The higher the phenolic or flavonoid content in the plant, the higher the antioxidant activity (Septiana & Simanjuntak 2018). The provision of antioxidants and components of phenolic compounds can reduce oxidative stress, increase insulin sensitivity by improving insulin performance, and lessening free radicals formation. The active flavonoid compound in *Gurmara* plant extract could reduce glucose levels by increasing insulin levels (Shewamene *et al.* 2015; Widowati 2008). Moreover, isoflavones from soybean extract were able to improve insulin resistance and decrease glucose levels (Liu *et al.* 2010).

These results confirmed that the ethanol extract and its fraction of parijoto fruit contained flavonoid compounds that could reduce glucose levels in vitro. Flavonoids are one of the phenolic compounds that have a glucose-lowering effect. (Rao *et al.* 2010) stated that flavonoids from *Lantana camara* and *Combretum micranthum* also showed anti hyperglycemic in DM type I and type II. (Prashanto *et al.* 2011) and (Tapas *et al.* 2008) revealed that quercetin also could increase pancreatic regeneration and insulin release in streptozotocin-induced mice. Further studies are required to evaluate the pharmacological effect of the parijoto fruit ethanol extract and its fraction to the glucose level.

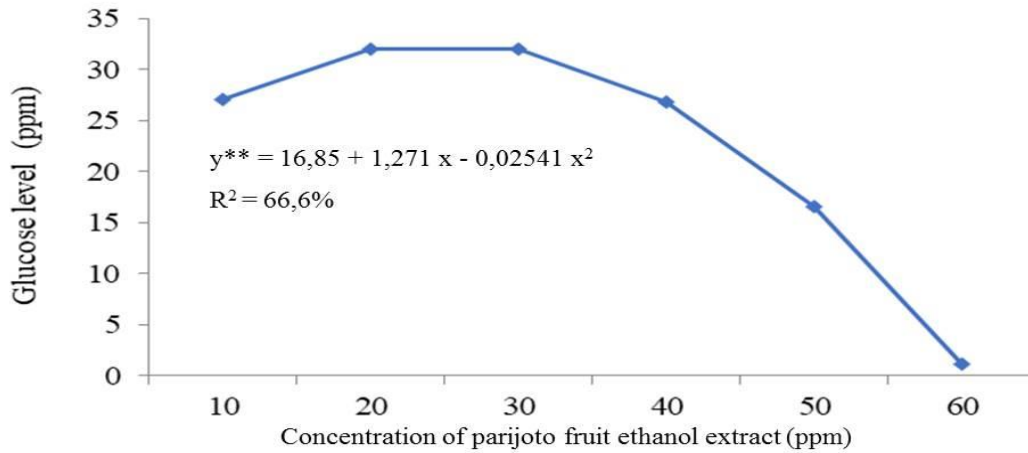


Figure 3. Correlation of glucose levels and concentration of parijoto fruit ethanol extract.  
Gambar 3. Korelasi antara kadar glukosa dan konsentrasi ekstrak etanol buah parijoto.

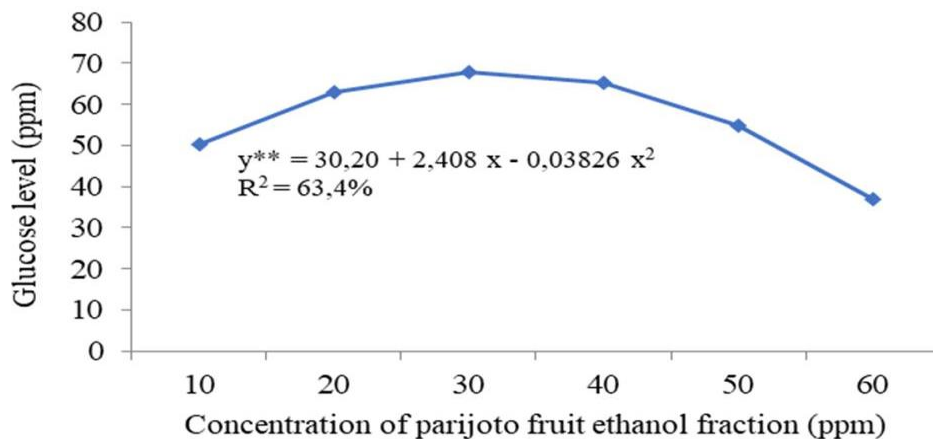


Figure 4. Correlation of glucose levels and concentration of parijoto fruit ethanol fraction.  
Gambar 4. Korelasi antara kadar glukosa dan konsentrasi fraksi etanol buah parijoto.

## CONCLUSIONS

Based on qualitative identification, the ethanol extracts and its fractions of the parijoto fruit contained a flavonoid compound that was able to decrease glucose levels *in vitro*. The ethanol fraction has a better ability to lower glucose levels compared to its ethanol extract. However, further research needs to explore for proving parijoto fruit as the antidiabetic candidate.

## ACKNOWLEDGMENTS

The authors want to acknowledge the Ministry of Research, Technology, and Higher Education for awarding Grant Fund for Beginner

Lecturer Research (PDP) for Fiscal Year 2018 with contract Number: 002/KTR-RESEARCH/DIKTI/LPPM/UNW/V/2018 to fund the research.

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